PRELIMINARY EVALUATION OF HIV 1 PROVIRAL DNA QUANTITATION ASSAYS

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ABSTRACT

Potent antiretroviral therapies that suppress cell-free plasma viral RNA levels below the limit of current assay detection necessitate other complementary approaches for assessing viral burden, such as quantification of cell-associated proviral DNA. A standard panel based on 8E5/LAV cells was established to facilitate the development and testing of several quantitative HIV 1 DNA assays. Five different assays were evaluated twice and three others once each. Our preliminary results suggest that HIV 1 proviral DNA could be measured precisely with a mean intra-assay standard deviation of 0.22 log10 DNA copies/106 cells (range, 0.14-0.40). However, it is unlikely that the measurement of total proviral DNA level alone will adequately describe the replicatively-competent infected cell pool. Thus, other virological markers, together with immunological markers, are necessary to provide a more complete laboratory based assessment of disease progression.

INTRODUCTION

According to recent guidelines, the quantification of human immunodeficiency virus type-1 (HIV 1) RNA is the cornerstone for monitoring the effectiveness of antiretroviral therapy [1-3] . For the majority of therapy naive patients, three to six months of potent antiretroviral therapy usually suppresses cell-free HIV 1 RNA levels to less than 50 copies/mL of plasma [4]. However, despite this successful suppression of viral RNA, there is substantial evidence to indicate ongoing low-level viral replication in the infected patient [5]. Consequently, there is an opportunity for continued genetic evolution of the virus with the development of drug resistance and eventual escape from viral containment. Without a further improvement in the laboratory's ability to practically quantify the level of plasma viral RNA, other methods for defining the containment of viral infection must be sought. Moreover, combinations of different laboratory methods for assessing HIV 1 replication may lead to a better understanding of viral pathogenesis following antiretroviral therapy.

In this regard, there is a practical interest in complementing viral RNA level with a more precise quantitative assessment of the circulating cell-associated proviral DNA pool. Thus, a working group was established under the auspices of the AIDS Clinical Trial Group and the NIAID-sponsored Virology Quality Assurance Program, to develop reagents and compare several assay methods for quantifying proviral DNA. On behalf of our working group, I shall present an interim report on our progress to date.

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MATERIALS & METHODS

A standard panel of 8E5/LAV cells was established to facilitate the development and testing of these assays. Five different assays were evaluated twice and three others once each (Table 1). Total proviral DNA was quantified using either external (N = 1) or internal standards (N = 7) and limiting dilution or quantitative competitive assay designs. Either direct cell count (N = 6) or total cell DNA quantification (N = 2) were used to establish the reporting denominator. Total HIV 1 proviral DNA was also measured using a panel of infected whole blood specimens obtained from three HIV 1 infected patients and an uninfected patient control.

RESULTS

The estimates of proviral copy number were indistinguishable among the estimates from 5 out of 8 assays for samples containing nominal concentrations of 32, 100, 320 and 1000 DNA copies/million cells (estimated vs. nominal correlation, r2 range, 0.73-0.96). The median estimates of proviral DNA for four replicates each from three HIV 1 infected patient blood samples were 1015, 409 and 500 DNA copies/106 peripheral blood mononuclear cells (PMBC) and 305,174 and 133 DNA copies/g DNA. There was greater intra-assay variation among the patient samples (median (SD) 0.26, range 0.08-1.32 log DNA copies/106 PBMC) than among the standard panel specimens (SD 0.22, range 0.14-0.40). There was also greater concordance between the two laboratories that reported results as DNA copies/&g of cell DNA.

DISCUSSION

Our preliminary results suggest that HIV 1 proviral DNA can be measured precisely using different assay formats. The wide range in intra-assay variation may have been caused by differences in cell counting methods and assay formats. The working group is investigating the effect of cell counting versus direct quantification of total DNA for the reporting denominator. As with other assays, eventual implementation of a quality assurance program to assess the performance of quantitative proviral DNA assays will be needed for clinical study comparisons.

The presence of cell-associated HIV 1 DNA provirus reflects, in part, the reservoir of infected cells. However, the majority of proviral DNA exists as either partially or completely reverse-transcribed unintegrated

DNA, which is considered replicatively incompetent [6]. A small percent of the proviral DNA is integrated and of this, only a proportion represents replicatively competent provirus. Thus, only a small percent of the total proviral DNA is actually available as a template for viral replication (Figure 1).

Moreover, when viewing retroviral replication in total, probably less than 10 percent of viral-directed transcription and translation results in replicatively competent virions capable of establishing new rounds of infection. Thus the majority of circulating plasma-associated virus is defective [7-9] . From both a pathogenesis and patient management perspective, these nuances of retroviral replication are germane for understanding those viral parameters that reflect the replication competency of the virus. As a consequence, the measurement of total proviral DNA alone will not adequately describe the replicatively competent infected cell pool. The direct quantification of integrated provirus, other proviral DNA moieties such as the unintegrated circular 2-LTR provirus, or direct measurements of viral transcriptional activity in the form of spliced and unspliced viral RNA, will then likely be useful supplements to the measurement of total provirus level [10].

In summary, despite the limitations imposed by retroviral replication, the majority of current therapeutic decisions are based on sampling from the pool of circulating viral RNA for either

direct quantification or drug susceptibility genotype or recombinant gene phenotype [3, 11, 12]. It is somewhat problematic that this intensively analyzed pool of viral RNA is only a partial surrogate marker of clinical outcome [13, 14]. Clearly, other virological markers, together with immunological markers, are necessary to fully characterize the pathogenesis of HIV 1 infection and provide a more complete laboratory-based assessment of disease progression.

Table 1

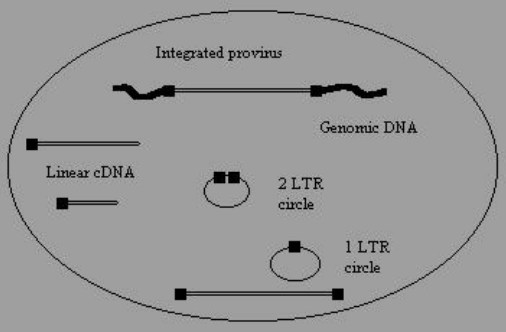
Table 1. Description of the eight different assay formats used by the working group laboratoriesLaboratory

Identification Code	Assay Format	Denominator for reporting HIV 1 DNA copy number
A	Amplicor™-LD	/PBMC*
B	Diagene-EQS	/PBMC*
C	QC-PCR-EIA	/µg or PBMC†
D	Amplicor™-LD-ML	/PBMC*
E	Amplicor®-LD-X²**	/PBMC*
F	PCR-EIA-IQS	/PBMC*
G	Amplicor™-LD	/PBMC*
H	Amplicor™-IQS	/µg or PBMC*

Abbreviations used: LD=limiting dilution; ML=maximum likelihood; QC=quantitative-competitive; IQS=internal quantitative standard; EQS=external quantitative standard; PBMC=peripheral blood mononuclear cells.
**minimum chi-square method
† calculated from DNA content of diploid human genome (4.28 x 10^{12} g/mole) = 1 µg/150,000 cells
* assumed a cell count (5 x 10^6 cells/pellet) calculated by the VQAP Laboratory.

Figure 1

Figure 1. Schematic of the various reverse-transcribed HIV-1 DNA provirusmoieties that may be found in the infected cell's nucleus. After attachment of the virus to the specific CD4-co-receptor plasma membrane complex, the reverse transcription of the two single-stranded viral RNA copies to double-stranded linear cDNA occurs in a cytoplasmic-associated structure derived from the nucleocapsid, which is followed by nuclear localizatio of the cDNA(not shown). The linear form of cDNA is the important intermediated for integration. Partially reverse-transcribed moieties and the 1-and 2-LTR circular forms represent aberrant reverse transcripts that are incapable of integration; however, their levels may provide insight into the molecular determinants of viral infectivity and disease pahtogenesis [5, 6, 10, 14].



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